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Volume 24 | Issue 1 Article 5

1962

Practical Diagnostic Bacteriology

Thomas M. Ford
Iowa State University

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Thomas M. Ford, D.V.M., M.S.*

Although there is a growing awareness of the value of diagnostic bacteriology by practicing veterinarians, the benefits that can be gained by practical diagnostic methods are often unutilized. Perhaps one reason practitioners shy away from using bacteriology as a diagnostic tool is because of the misconception that the identification of pathogenic organisms is extremely complex and requires a vast amount of specialized equipment. It is hoped that this discussion will arouse further interest in diagnostic bacteriology and show its applicability to the veterinarian's office.

A suggested list of media and equipment for an office bacteriology laboratory would include the following items:

Equipment.
- Microscope
- Incubator
- Autoclave or pressure cooker
- Bunsen burner or hand torch
- Balance
- Inoculating loops, spatula, staining rack, etc.

Glassware.
- Glass microscope slides and coverslips
- Pyrex petri dishes (disposable plastic petri dishes may be substituted)
- Screw cap and pyrex test tubes
- Graduated cylinder (100ml) and pipettes
- Miscellaneous flasks and beakers

Media.
- Blood agar base
- MacConkey's agar
- Kligler's iron agar
- Urease agar
- Sabouraud's agar
- Sodium or potassium hydroxide
- Antibiotic sensitivity discs

Stains.
- Gentian violet solution
- Gram's iodine solution
- Safranin solution
- Acetone-alcohol solution
- Lacto-phenol cotton blue

This is merely an arbitrary list and additions or deletions can be made according to individual needs. It is difficult to give a cost estimate for this equipment as some items, such as an incubator, can be homemade, while the cost of other items, such as a microscope, will depend upon the quality desired.

The first and foremost factor in diagnostic bacteriology is the proper collection of the sample. Extreme care must be taken to obtain the best specimen possible or culturing will be a waste of time from the beginning. This fact cannot be overemphasized, for isolation and identification of the pathogen from a badly contaminated sample is extremely difficult and often impossible. This is particularly true in the case of milk samples taken for mastitis diagnosis.

One of the most satisfactory methods for obtaining samples from body surfaces and exudates is by the use of sterile cotton-tipped swabs. These are prepared by inserting the swab into a test tube and plugging the tube with a cotton plug. An appropriate number of swabs is then sterilized in an autoclave or pressure cooker and stored for further use. Sterile swabs

* Dr. T. A. Ford is an Instructor in the Department of Veterinary Hygiene, Iowa State University.

may also be purchased in disposable plastic tubes from surgical supply houses.

A few basic rules are involved in obtaining a good specimen for diagnosis. When a swab is to be applied to a body surface the area should first be thoroughly cleansed to reduce the large number of surface contaminants. After the swab is touched to the affected part it should be immediately replaced in the sterile tube.

Specimens from autopsy tissues can be obtained by the use of swabs or an inoculating loop. The surface of the tissue is seared with a hot spatula, or by the flame of a bunsen burner, and the area opened with a flamed scalpel. The sterile swab or loop can then be inserted directly into the incision.

Milk samples taken for mastitis diagnosis should be taken with care. Wash the ventral side of the udder with one of the common dairy antiseptics (such as B·K Powder) using a clean towel. Particular care should be taken with the teat orifice. Milk 4 to 5 ml into a sterile glass vial, being careful not to contaminate the open end of the vial. Quickly replace the cork or rubber stopper and incubate the milk samples for 12 to 18 hours at 37° C. After this time the milk can be stained for the presence of bacteria and cells, either by the Gram's stain or the Newman-Lambert staining technique. The milk can also be streaked on blood agar plates at this time.

After proper collection of the sample, the next step is to transfer the specimen onto the surface of a suitable agar medium. The swab is rubbed over a small area of the agar near the edge of the plate and a flame-sterilized inoculating loop is used to streak the material out onto the remaining areas of the plate. When dealing with fluids such as milk or urine the procedure is the same except a loopful of the fluid is used rather than a swab. The object of streaking is to scatter out individual organisms thinly enough so they will grow up into isolated colonies. It is imperative when transferring growth from a colony to another media, or to a slide for staining, that only one colony is picked, so as to insure a pure culture. Never touch more than one isolated colony with the transfer loop.

Before discarding the swab it is a good procedure to streak a clean slide and make a Gram's stain of the material. This initial microscopic examination of the material is often important in evaluating the growth obtained on the solid media. Precautions should be taken that there are no antibiotics present in the collected sample as this will almost invariably produce sterile cultures.

A blood agar plate is usually the medium of choice for initial isolation of pathogenic organisms. One exception is when feces or tissues are being cultured for enteric organisms or when a Gram negative organism that cannot be identified as *Pasteurella* or *Pseudomonas* is isolated on blood agar. In this case a MacConkey's agar plate should be used. This medium prevents the growth of all organisms except the enterics, which are all Gram negative rods. The lactose-fermenting coliforms (*Escherichia coli, etc.*) can be easily differentiated from the non-lactose fermenting organisms (*Salmonella, Proteus*) by the color of the colonies. The lactose fermenting organisms grow as pink colonies, while the non-lactose fermenting colonies are colorless on MacConkey's agar.

Since the genus *Salmonella* is of primary concern when enterics are encountered, steps should be taken to differentiate it from a possible *Proteus*. This is done by inoculating a urease agar tube with the organisms and incubating for 4 to 5 hours at 37° C. *Proteus* will turn the medium red while *Salmonella* will produce no color change. Further information about the biochemical properties of the organism can be obtained by observing the reactions on Kligler's iron agar. Consult a standard textbook or the Difco Manual (available from Difco, Inc., Detroit, Michigan) for the method of inoculation and explanation of reactions on this media. Also, if the genus *Salmonella* is suspected and not found on primary isolation with MacConkey's agar, consult the literature on Selenite F broth in Difco's Manual.

Another exception where blood agar plates are not used for primary isolation is when a fungus infection is suspected (us-

ually ringworm). Here an antibiotic-containing Sabouraud's agar is used which allows only the growth of pathogenic fungi. Unlike the previously mentioned media which are incubated at 37° C. for 24 to 48 hours, Sabouraud's agar plates are incubated at room temperature for 5 to 14 days. Growth from this medium is then mounted in a drop of lacto-phenol cotton blue, coverslipped, and observed microscopically for the presence and shape of spores and mycelia.

An initial examination of the suspected material can be made by taking skin or hair, aseptically, from the periphery of the lesion. Place it on a slide in a drop of 10% sodium or potassium hydroxide, heat it gently, and then allow it to stand for several minutes to clear. It is then similarly examined for spores and mycelia. There are usually only two genera of fungi associated with ringworm, namely, *Trychophyton* and *Microsporum*. These can be recognized by comparing gross and microscopic characteristics of isolates with a standard text.

In summary, therefore, the three essential media necessary for primary isolation are:

1. *Blood agar* — for most routine specimens, including mastitis milk.
2. *MacConkey's agar* — for isolation of enterics. Urease agar and Kligler's iron agar may be used for further identification.
3. *Sabouraud's agar* — for culturing fungi.

Gross observation of isolated colonies on blood agar plates (size, surface characteristics, pigmentation, hemolysis, etc.) together with a Gram's stain of the colony will often enable immediate identification of the organism. Many organisms, however, do require further procedures for identification, such as the enterics and fungi which were mentioned above. A brief discussion of the more commonly encountered animal pathogens may help in their identification.

I. COCCI

No Gram negative cocci are recognized as pathogens in veterinary medicine. Of the Gram positive cocci, only two genera are animal pathogens, *Staphylococcus* and *Streptococcus*.

A. Gram Positive Cocci.
   1. *Staphylococci* — grow well on blood agar producing smooth, glistening, medium-sized colonies with a characteristic double zone hemolysis (an area of complete clearing immediately around the colony and an outer ring of partial clearing). Gram stained smears will show the Gram positive cocci in clumps (grape-like clusters).
   2. *Streptococci* — grow well on blood agar producing pin-point, glistening dew-drop like colonies. Colonies often produce clear or greenish hemolysis. Gram stained smears show Gram positive cocci in chains of 2 to 6 or more organisms.

II. RODS

A. Gram Positive Rods.
   1. *Corynebacteria* — form small dew-drop colonies on blood agar, similar to *Streptococci*. Zones of hemolysis usually do not appear until 48 hours after incubation. On a Gram stained smear the organism appears as a small coccoid, pleomorphic rod. The ends are often club-shaped or pointed. Palisading is often seen. *Corynebacterium pyogenes* is the primary pathogen of this genus. However, many diphtheroids exist as skin contaminants.
   2. *Bacilli* — common contaminants, producing large, irregular gray colonies with marked hemolysis. Stained smears show large Gram positive rods, often in chains. *Bacillus anthracis* is the only pathogen considered in this genus. It is non-hemolytic and if suspected should be submitted to a diagnostic laboratory for further identification.

B. Gram Negative Rods.
   1. *Enterobacteria* (*Escherichia, Salmonella, Proteus*) — produce gray, smooth, glistening, non-

hemolytic colonies on blood agar. *Proteus vulgaris* is characterized by swarming colonies. Stained smears show Gram negative short, plump rods.

2. *Pasteurella*—colonies produce variable hemolysis or a slightly green discoloration of the blood agar. Stained smears show Gram negative rods with characteristic bi-polar staining.
3. *Pseudomonas* — produces distinct hemolysis on blood agar, has a characteristic sweetish odor and often produces a greenish pigment (hard to detect on blood agar). Colonies are large, grayish and irregular. Stained smears reveal Gram negative rods.

This list covers the majority of bacteria that can be readily identified with a minimum amount of time and equipment. There are many other pathogens of great importance in veterinary medicine, such as *Listeria, Vibrio,* the *Clostridia* and *Leptospira,* which require more specialized procedures for their identification. If these organisms are suspected it is best to rely on the services of a diagnostic laboratory.

Determination of susceptibility of microorganisms to various antibiotics can be easily carried out with antibiotic sensitivity discs. The test is performed on plates of media inoculated with the isolated organism in pure culture. Do not run the sensitivity test directly on the material being cultured since so many contaminants will be encountered that the results would be meaningless. Use only a pure culture of the organism.

The procedure is as follows: An isolated colony of the organism to be tested is picked up with a sterile swab. Dipping the swab in a tube of sterile saline, after picking the colony, will facilitate even distribution of the organism when smeared on a blood agar plate. The antibiotic discs are then placed on the surface of the media and the plate incubated at 37° C. for 24 hours. A clear zone free of growth around a disc indicates that the antibiotic inhibited growth of the bacteria. A common misunderstanding about sensitivity tests is that the larger the zone of inhibition, the more effective the antibiotic. This is not necesarily true, since such factors as diffusibility of a particular antibiotic in an agar medium will influence the relative size of the inhibition zone. Readings, therefore, should simply be made as resistant or sensitive to the particular antibiotic. Another fact to remember is that sensitivity tests for sulfa drugs should not be run on blood agar plates. The para-amino-benzoic acid in the blood will inactivate the sulfa so that the organism will always appear resistant to the drug.

It is hoped this discussion of diagnostic bacteriology has demonstrated that there are many pathogenic microorganisms which can be readily identified with a minimum of effort. The employment of these procedures cannot help but be of assistance in the diagnosis of disease, and in carrying out the best course of treatment.

Homecoming Luncheon

The thirteenth annual Veterinary Homecoming Luncheon was held in the Veterinary Quadrangle. The luncheon was to be in the courtyard but due to poor weather it was held in the auditorium. A tasty meal of baked beans, ham and potato salad was served. Ice cream was furnished for dessert. Approximately 450 people attended, and afterwards many saw Iowa State play Kansas State in the Homecoming game.